HiPSC-derived Neural Stem Cells (HiPSC-NSC)

Catalog #1650

Cell Specification

Human Neural Stem Cells (Cat. #1650) from ScienCell Research Laboratories are differentiated from a human induced pluripotent stem cell line (hiPSC), which is generated using mRNA reprogramming technology from Human Dermal Fibroblasts (HDF). The monolayer HDF-hiPSC are efficiently converted to neural epithelium using HPSC Neural Induction Medium (PSCNIM, Cat. #5931), a serum-free medium for rapid and efficient neural induction of human pluripotent stem cells (hPSCs). Synergistic inhibition of glycogen synthase kinase 3 (GSK3) and transforming growth factor β (TGF-β) differentiates HDF-hiPSCs to homogenous neural stem cells (NSC) in the presence of human leukemia inhibitory factor (LIF) within 7 days.

The derived NSC are characterized by immunofluorescence with antibodies specific to nestin and SOX2. The cell population is highly pure: >90% of cells express Nestin and >80% of cells are SOX2 positive. After reviving, NSC can be maintained in HPSC Neural Induction Medium as an adherent culture or form neural spheres in EGF and bFGF containing neural stem cell medium. The cells are able to form rosette and neural tube-like structures after being passaged and plated at high density on Matrigel™ coated culture vessels in neural stem medium containing EGF and bFGF. HiPSC-NSC can be further expanded for months as a monolayer on Matrigel™ coated culture vessels or in suspension as neural spheres when seeded on ultra-low binding culture vessels in neural stem cell medium. NSC are multipotent and able to differentiate into various neuronal and glial subtypes. Specific patterning cues, such as SHH, retinoic acid and FGF8, can be added after reviving to direct the cells to different neural lineages.

HiPSC-NSC are cryopreserved at passage 0 and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. Cells are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

Product Content

1 vial of frozen HiPSC-NSC (Cat. #1650, 1 mL, >1 x 10^6 cells/vial)
50 mL Neural Induction Medium-basal (PSCNIM, Cat. #5931-50)
1 mL Neural Induction Medium Supplement 50x (PSCNIS, Cat. #5982-50)

Recommended Medium

It is recommended to use the provided Neural Induction Medium (PSCNIM, Cat. #5931) for plating HiPSC-NSC and expanding them in the short term. Adding ROCK inhibitor Y-27632 in the first 24 hours after reviving improves cell viability and attachment in adherent cultures.

Other neural stem cell medium containing bFGF and EGF can be used for reviving and long term expanding of the cells.

To form neural spheres, ultra-low binding culture vessels and neural stem cell medium containing bFGF and EGF are recommended.

To differentiate the HiPSC-NSC, medium containing specific growth factors should be used.

Additional Materials Required
For adherent culture:

- BD Matrigel™ hESC-qualified matrix (BD Biosciences, Cat. #354277)
- ROCK Inhibitor Y-27632 (Tocris Bioscience, Cat. #1254)

For suspension culture:

- Neural stem cell medium containing EGF and bFGF
- Ultra-Low Binding Culture Plate (Cat. #0383)
- ROCK Inhibitor Y-27632 (Tocris Bioscience, Cat. #1254)

Product Use
HiPSC-NSC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage
Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping
Dry ice.

References
[1] Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. (2009) "Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling." *Nat Biotechnol*. 27(3): 275-280.
[2] Li W, Sun W, Zhang Y, Wei W, Ambasudhan R, Xia P, Talantova M, Lin T, Kim J, Wang X, Kim W, Lipton SA, Zhang K, Ding S. (2011) "Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors." *PNAS*. 108(20): 8299-8304.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37^{o}C water bath and return them to culture as quickly as possible with minimal handling!

Note: HiPSC-NSC are very sensitive cells and they can proliferate many times before becoming terminally differentiated if cells are grown using the following protocol. The following procedures are optimized for 12-well plates and indicated volumes are for one well of a 12-well plate.

Initiating the culture as an adherent culture:

1. Prepare BD Matrigel^{TM}-coated 12-well plates according to the manufacturer's instructions and warm to room temperature before using.
2. Prepare complete Neural Induction Medium (PSCNIM): thaw the 50x supplement at room temperature; decontaminate the external surfaces of medium bottle and supplement tube with 70% ethanol and transfer them to a sterile field. Aseptically open the supplement tube and add to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.
3. Warm the medium to room temperature prior to thawing the cells. Aliquot 10 mL of the medium into a 15 mL conical tube and leave it in the hood.
4. Take one vial of neural stem cells out of the liquid nitrogen. Immediately transfer the vial into a 37^{o}C water bath and gently swirl it for 90 seconds or until most of contents are thawed and only a small piece of ice remains.

   Note: The viability of the cells will decrease if the vial contents are completely thawed.
5. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 2 mL pipette, gently resuspend the contents of the vial and transfer to the 15 mL conical tube containing 10 mL of the medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: Minimize the time for step 5-6.
6. Centrifuge the tube at 1000 rpm for 5 minutes at room temperature.
7. Aspirate supernatant carefully. Be careful not to disturb the cell pellet.
8. Tighten the cap of the tube and loosen the cell pellet by tapping the bottom of the tube. Add 2 mL of the neural induction medium into the tube and mix well. If a large visible cell pellet is present, try to break them into small pieces by gently pipetting 2 - 3 times with a 5 mL pipette.
9. Add ROCK inhibitor Y-27632 to the cell suspension at 5 µM and mix well.

   Note: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.

10. Bring the Matrigel™ coated plate to the hood and aspirate the Matrigel™ from the well. Add 2 mL of cell suspension into the well. Replace the cover and gently rock the vessel to distribute the cells evenly.

    Note: The recommended cell seeding density is 2.5 – 5 x 10^5 cell/cm^2.

11. Return the culture vessel to the incubator.

12. For best results, do not disturb the culture for 24 hours after the culture has been initiated. Change the medium the next day to remove unattached cells, then every other day thereafter.

Initiating the culture as a neural sphere culture:

1. Warm the neural stem cell medium containing EGF and bFGF to room temperature prior to thawing the cells. Aliquot 10 mL of the medium into a 15 mL conical tube and leave it in the hood.

2. Take one vial of neural stem cells out of the liquid nitrogen. Immediately transfer the vial into a 37°C water bath and gently swirl it for 90 seconds or until most of contents are thawed and only a small piece of ice remains.

   Note: The viability of the cells will decrease if the vial contents are completely thawed.

3. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 2 mL pipette, gently resuspend the contents of the vial and transfer to the 15 mL conical tube containing 10 mL of the medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: Minimize the time for step 5-6.

4. Centrifuge the tube at 1000 rpm for 5 minutes at room temperature.

5. Aspirate supernatant carefully. Be careful not to disturb the cell pellet.

6. Tighten the cap of the tube and loosen the cell pellet by tapping the bottom of the tube. Add 2 mL of the neural stem cell medium into the tube and mix well. If a large visible cell pellets is present, try to break them into small pieces by gently pipetting 2 - 3 times with a 5 mL pipette.

7. Add ROCK inhibitor Y-27632 to the cell suspension at 5 µM and mix well.

   Note: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.

8. Bring an ultra-low binding plate to the hood and add 2 mL of cell suspension into one well. Replace the cover and gently rock the vessel to distribute the cells evenly.

9. Return the culture vessel to the incubator.

10. For best results, do not disturb the culture for 24 hours after the culture has been initiated. Small neural spheres should appear on the next day.

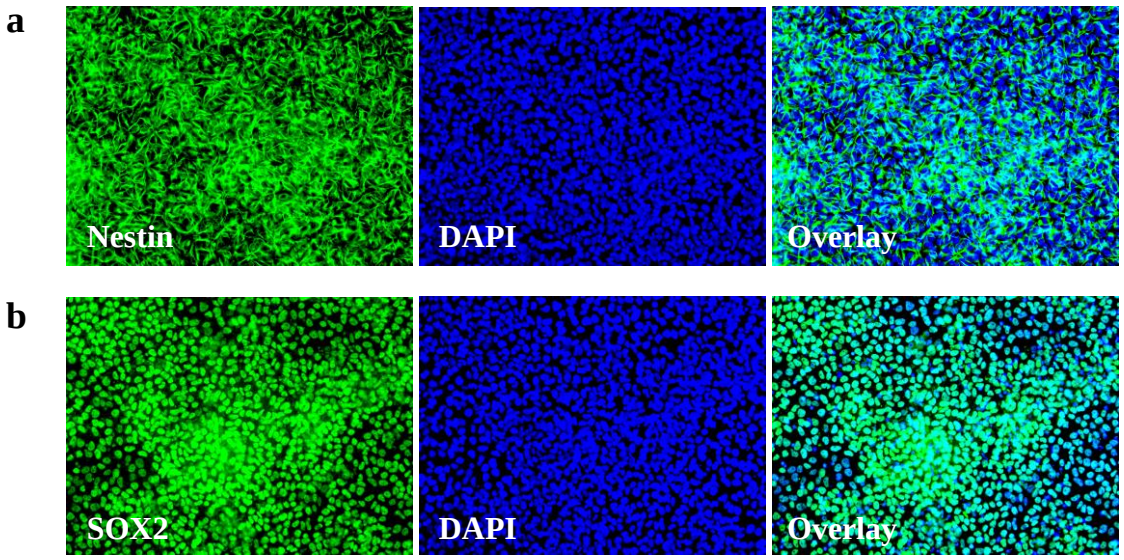
    We recommend Neural Induction Medium (PSCNIM, Cat. #5931) to revive HiPSC-NSC and culture the cells adherently at a high density for the short term. HiPSC-NSC can be revived or further expanded in neural stem cell medium containing bFGF and EGF, either as an adherent culture or as neural spheres for long term culture.

For neuronal differentiation, cells differentiate into neurons in 10-15 days after withdrawing the growth factors. Cells maintained in the EGF and bFGF medium for more than 60 days can efficiently differentiate into astrocytes. To differentiate cells to a specific neural type, patterning factors need to be applied early after reviving.

Caution: Handling human-derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

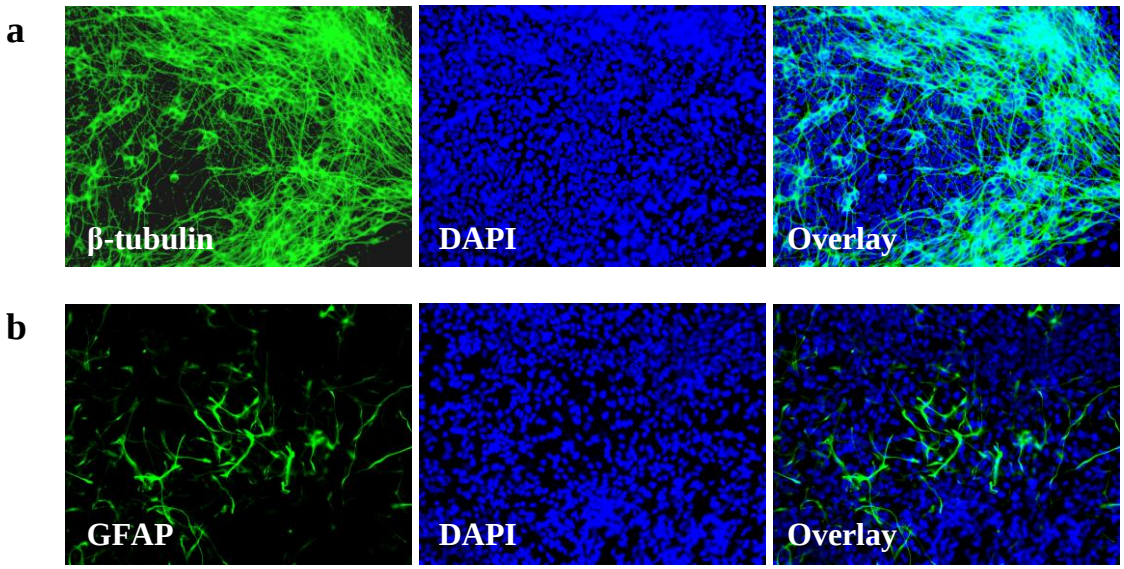
[1] Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11(4).

Figure 1. Revived HiPSC-NSC express neural stem cell markers.



The revived HiPSC-NSC were characterized by immunostaining with antibodies against Nestin (a, green) and SOX2 (b, green). Nuclei were stained with DAPI (blue).

Figure 2. hiPSC-NSC are able to differentiate into neurons and astrocytes.



hiPSC-derived NSC can differentiate into neurons upon growth factor withdrawal (a, β-tubulin, green). After maintaining cells in the EGF and bFGF containing medium for > 60 days, cells can differentiate into $GFAP^{+}$ astrocytes (b, GFAP, green). Nuclei were stained with DAPI (blue).